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The Role of Free Radicals in Tumor Promotion

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A role has been suggested for free radicals and active states of oxygen in tumor promotion. There are a number of lines of support for this hypothesis, but no definitive evidence. The hypothesis has proven of value in leading to the development of models pertinent to understanding the mechanism of action of tumor promoters.

Tumor promotion is a complex process of importance to the development of cancer. Since our original suggestion along with Walter Troll's in 1980 of the potential role for radicals in tumor promotion, much has been written on this subject. At present, it appears not unreasonable that free radicals or active states of oxygen may play an important role in one or more of the stages of tumor promotion.

The work I am about to describe would not have been possible without the help of Norton Nelson. I say this despite the fact that I cannot recall a specific discussion of this work with him, nor can I point to any part of the hypothesis generation, or of the research in which he participated directly. However, none of it would have been possible without Nelson's ability to develop the appropriate ambience, provide the support structure, serve as a role model, and just generally make things happen. What I will describe is a collaborative research project among three very dissimilar scientists. While Nelson did not assign us a project on which to collaborate, nor tell us we must collaborate on anything at all, nevertheless, our collaboration was not accidental. It was a product of his understanding of the scientific process coupled with his unsurpassed organizational skills.

Scientists at the NYU Institute of Environmental Institute have long had an interest in tumor promotion, developing many of the central concepts of this interesting and important mechanism of carcinogenesis. Walter Troll, a biochemist, has been one of the leading authorities in this area and has brought to it his particular knowledge about proteolytic enzymes and antiproteases. Gisela Witz is a superb organic chemist who worked on a number of tumor promotion projects in the laboratory of Benjamin Van Duuren. My own training is that of a hematologist with a primary interest in free radical reactions occurring in cellular membranes. Although I was not directly involved in any studies of carcinogenesis, my growing enthusiasm about the potential role of free radicals in a whole host of biological and toxicological processes led me to challenge others in the department to consider the potential role of these active species in chemical carcinogenesis, including tumor promotion. Witz joined my laboratory to collaborate in studies aimed primarily at measuring cell membrane fluidity as an early marker of the potential effects of free radicals and of lipid peroxidation decomposition products.

Fortunately for us, Nelson's administrative alchemy put us in laboratory space which we shared with Walter Troll. One of the interests in our laboratory was the then recently described production of superoxide anion radical by stimulated phagocytic cells, a process that appeared necessary for bacteriocidal activity. The presence of an obligatory lag period between the addition of a stimulus and the eventual burst of oxygen consumption leading to free-radical formation suggested the possibility that a proteolytic mechanism may be involved. While working with Troll and Marie Amoruso, we were able to demonstrate that a variety of antiproteases acted in a specific manner to inhibit the burst of oxygen consumption from different phagocytic cell types and were induced by both soluble and particulate stimuli (*1*).

One of the most potent stimulators of active oxygen consumption in the production of free radicals by phagocytic cells was known to be the prototypic tumor promotor phorbol myristate acetate (PMA). Futhermore, Troll's

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studies showed that many of the same protease inhibitors that prevented the production of superoxide anion radical from phagocytic cells were also potent inhibitors of tumor promotion in the mouse skin system (*2*). This quite naturally led us to agree that the hypothesis that free radicals could be involved in tumor promotion was worthy of study and that a logical source of such radicals in the mouse skin system could be inflammatory cells (*3, 4*). This hypothesis is shown in Figure 1.

Of note is that the then known tumor promoters in the classic two-stage mouse skin system were all inflammatory agents and that inflammation had been suggested as having a role in human cancer causation at least as early as the nineteenth century. However, prior studies had been unable to correlate tumor-promoting ability with inflammation, as measured by standard techniques such as number of infiltrating inflammatory cells, thus appearing to preclude a role for inflammation in tumor promotion. Yet many clinical observations suggested a role for chronic inflammation in tumor development, observations ranging from basal cell tumors on the bridge of the nose of eyeglass wearers, to colonic cancer in patients with ulcerative colitis.

In our studies we found that in contrast to other measures of inflammation, the rate of $\cdot O_2$ production in human polymorphonuclear leukocytes (PMN) was well correlated with the tumor-promoting activity of the phorbol esters (*5*). Mezerein and teleocidin B were slightly better stimulators of $\cdot O_2$ production than was PMA. Acetic acid was inactive (*6*). We tested various retinoid derivatives for their ability to inhibit PMA-stimulated $\cdot O_2$ production by human granulocytes, as they also had been reported to interfere with tumor promotion (*6*). A dose-responsive inhibition was observed with all-*trans* retinol, retinyl acetate, and retinoic acid (*7*). Preliminary evidence that PMA produced hydrogen peroxide in mouse skin was also obtained (*3*).

Our hypothesis was not limited to macrophages as a source of free radicals. For example, hepatic models of tumor promotion tend to use compounds that are powerful inducers of cytochrome P-450 or of peroxisomes, both conceivably sources of free radicals or other active states of oxygen. Of note is that Cattley and Popp recently have presented evidence that the potent peroxisome proliferator WY-14,643 acts as a promoter, and not as an inducer, in producing liver cancer (*8*).

Recent studies by Witz and Cziernicki have further addressed the question of whether free radicals generated from inflammatory cells might be responsible for tumor promotion *in vivo*. A model system has been developed in which murine peritoneal macrophages are treated *in vivo* with tumor promoters and subsequently assessed *in vitro*, allowing exploration of the *in vivo* production of active states of oxygen (*9, 10*).

This has proven to be a very useful system to investigate the role of known tumor promoters. Among the findings has been a different response to PMA, a complete promoter, as compared to mezerein, a second-stage promoter. Further, the identification in this system that phorbol diacetate (PdA) inhibited oxy radical production stimulated by mezerein suggested that PdA could affect second-stage promotion by this compound. This prediction from the mouse peritoneal system was confirmed in a bioassay (*9*).

Tumor promotion has of course been shown to be a relatively complex process with at least two stages. Free radicals and active states of oxygen may be particularly important in the second stage of promotion. Mezerein, which is as or more potent than PMA in stimulating macrophage oxygen consumption, is only active in the second stage of promotion (*11*).

The hypothesis that free radicals and active states of oxygen play a role in tumor promotion has led to a series of testable hypotheses and some interesting research findings from a number of laboratories (*12,13*). While the overall hypothesis remains far from proven, it is not at all unlikely that free radicals are involved in at least certain aspects of tumor promotion, including progression to frank carcinoma. It must be emphasized that cancer can be considered to be a final common biological pathway with many different routes, each with a multiplicity of steps, leading to a clinically recognized cancer. The question is whether free radicals or related active species and their products play a role in any of these routes leading to human cancer. It is distinctly unlikely that all, or even most, of these routes include a free radical step.

In conclusion, our studies implicate a role for free radicals and active states of oxygen in the sequence of events leading to the induction of tumors by promoters. They also indicate that the peritoneal macrophage system has the potential for being useful in the identification of promoters and inhibitors of promotion, as well as in mechanistic studies of the biochemical effects caused by promoters *in vivo*.

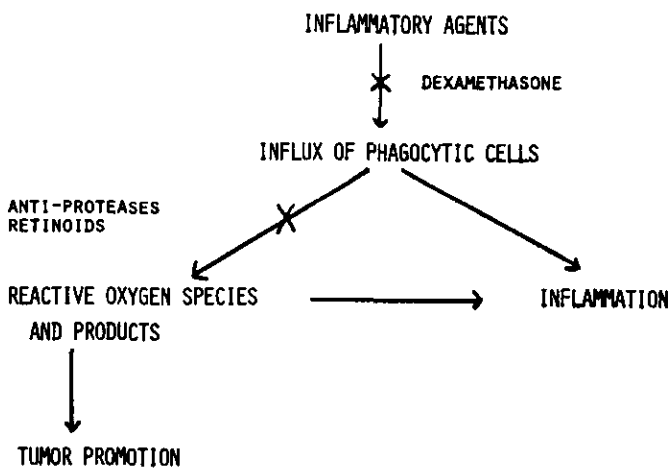

FIGURE 1. Hypothetical scheme for the involvement of reactive oxygen species in tumor promotion. Reprinted with permission (*3*).

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